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SYNTHESIS, CHARACTERIZATION AND ANTI-MICROBIAL ACTIVITY OF SOME NOVEL ALANINE DERIVATIVES OF RNA & DNA BASE

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ABSTRACT

Amino acid derivatives have been designed and synthesized with simple and eco- friendly methodologies with nucleobase. With this multivalent objective, the organic compounds were synthesized from reacting partner in solvent phase by microwave method. RNA & DNA base have been a very significant heterocyclic and also amino acids are having highly significant physiological activity. The structures of the compounds have been elucidated with the help of elemental analysis IR, Mass &^{13}C NMR spectral data. These novel synthesized compounds have been evaluated for their anti-cancer, anti-fungal activity and anti- bacterial activity.

Keywords: RNA & DNA bases, Alanine, Antibacterial activity, Antifungal activity.

1. Introduction

Amino acid and their derivatives of RNA & DNA bases were very important class of heterocyclic compounds.These derivatives were show various biological activities like antibacterial, antifungal, anticancer,anti-inflammatory activitiesGenerally nucleobases were also acts as a CNS stimulants and anti-oxidants.α-amino propionate which was non-essential amino acid.It was used in hypertension and diabetes.It was also utilized to increase the level of hypoglycemia and hepatitis. Because of these biological importance much attention was provided for the

synthesis of alanine derivatives of nucleobases. It was thought to synthesizesnew alanine derivatives and screen them for their biological activity.

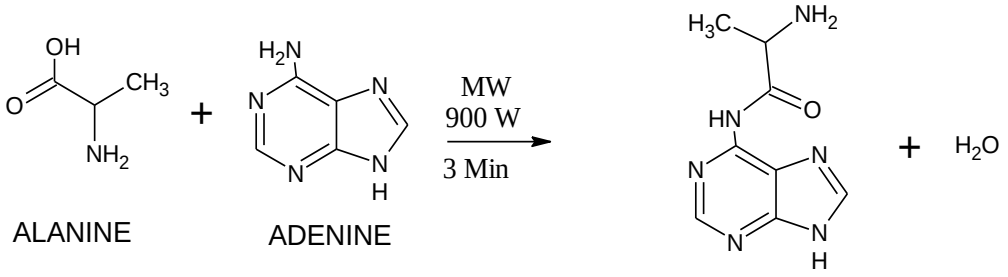
2. Materials and Method

The derivatives of amino acid had been carried out by reacted with various nucleobases. . This section deals with the preparation of alanine derivatives of nucleobases.

All the chemicals were used of analytical grade without further purification. Alanine, Adenine, Guanine, Thymine, Uracil, Ethanol, Distilled water, was used

General procedure For synthesis of various RNA & DNA base & amino acid derivative Alanine and RNA & DNA base were weighed equally in respect to the moles (0.02 : 0.02).The properly weighed compounds were thoroughly mixed using distilled water. The mixture of the compound was transferred into a RBF (250 ml).Then the RBF was place into microwave oven and set the microwave at full microwave radiation (900 W) as per reaction time and start the microwave oven. After the completion of reaction the RBF was takenfrom the oven very carefully. Then the reaction mixture was transferred into evaporating dish and evaporate the mixture and the product was collected. Recrystallize from hot water. When we were used guanine, the reaction was taken place in ethanol on behalf of water.

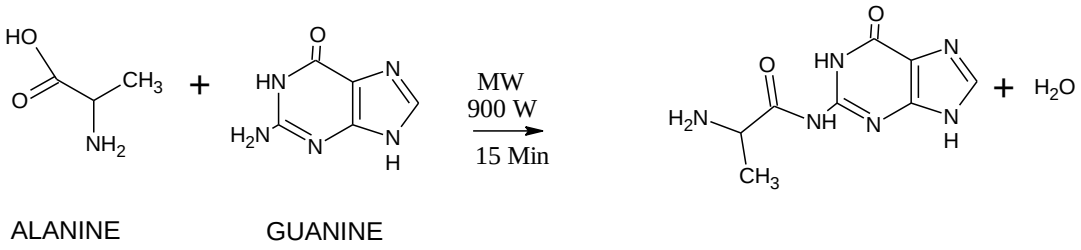
(1)Product M_1A (Alanine + Adenine)



ALANINE ADENINE

M_1A

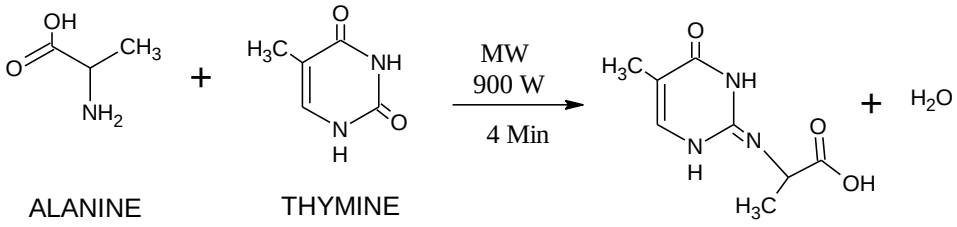
(2)Product M_1C (Alanine + Guanine)



ALANINE GUANINE

M_1C

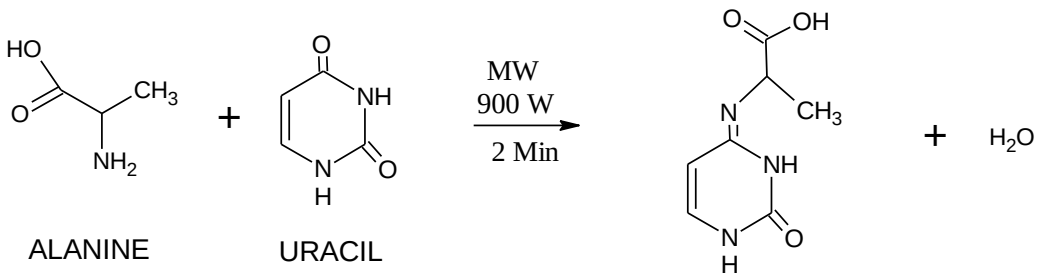
(3) Product M_1D (Alanine + Thymine)



ALANINE THYMINE

M_1D

(4)**Product M_1E (Alanine + Uracil)**



ALANINE URACIL

M_1E

3. Spectra Characterization

(1)Compound M_1A:

Infared Spectra Feature	1091, 1153, 1221, 1305: -C-N Stretch
cm^{-1}	1671 : -C=O Stretch (Amide)
1541, 1557 : -NH, $-NH_2$ bend	1508, 1596 : -C=C Aromatic Stretch

718 : -C-H Aromatic out of plane bend

3094 :-CH_3 Stretch

^{13}C spectral Features: (ppm)

40.09 ,39.88, 39.67, 39.46, 39.25, 39.04, 38.84: R_2-CH_2 , R_3-CH , C-N

(2) Compound M_1C

Infared Spectra Features :-

cm^{-1}

1558, 1507 : -$NH_,$ -NH_2 bend

[1045, 1116, 1148, 1173, 1213

1257, 1371] : -C-N Stretch

1669 :-C=O Stretch (Amide)

1748 : -C=O Stretch (Ketone)

1474 : -C=C Aromatic Stretch

778 : C-H Aromatic out of plane b

152.37 : R-CO-NH, C=O

138.08 : C=C

Mass spectral features

135.0 :Base peak is observed due to$C_5H_4N_5$.This is Adenine peak

3115 : - CH_3 Stretch

^{13}C spectral Features: (ppm)

40.11 ,39.90, 39.69, 39.48, 39.27, 39.06, 38.84 :R_2-CH_2 , R_3-CH, -C-N

Mass spectral features

149.1 : Peak is observed due to$C_5H_4N_5O$.Thisis Guanine peak.

135 .0 :Base peak is observed due to $C_5H_3N_4O$

(3) Compound M_3D

Infared Spectra Features :-

cm^{-1}

1540 : -$NH_,$ bend

1024, 1049, 1090, 1152, 1199 : -C-O Stretch (Carboxylic acid)

1748 : -C=O Stretch

1452, 1650 : -C=C Aromatic Stretch

806 : -C-H Aromatic out of plane bend

3029: - CH_3 Stretch

2359 – 2945 : -OH Stretch (Carboxylic acid)

1675 : -C=N Stretch

^{13}C spectral Features: (ppm)

11.74 : R-CH_3

40.01 ,39.80, 39.60, 39.39, 39.1, 38.97 ,38.76 : R_2-CH_2 , R_3-CH , C-N

164.90 ,151.47 :R-COOH, C=O

107.65 ,137.69 : C=C

Mass spectral features

197 .1 : Molecular peak is present. Peak is observed due to $C_8H_{11}N_3O_3$

148.1 : Peak is observed due to $C_7H_7N_3O$.

135.1 : Peak is observed due to $C_6H_7N_3O$.

126.0 : Base peak is observed due to$C_5H_6N_3O$..Thisis Thymine peak

112.1 : Peak is observed due to $C_5H_6N_2O$.

(4) Compound M_1E

Infared Spectra Features :- cm^{-1}

1650 : -NH_2 bend

[1049, 1049, 1153, 1148, 1233 , 1318, 1340] : -C-O Stretch (Carboxylic acid)

1746 : -C=O Stretch

1455, 1540 : -C=C Aromatic Stretch

818 : -C-H Aromatic out of plane bend

3118: - CH_3 Stretch

2360 – 2941 : -OH Stretch (Carboxylic acid)

1680 : - C=N Stretch

^{13}C spectral Features: (ppm)

40.02 ,39.82, 39.61, 39.40, 39.19, 38.98 ,38.77 :R_2-CH_2 , R_3-CH , C-N

164.33 ,151.50 : R-COOH,C=O

100.18,142.17 :C=C

Mass spectral features

184.1 : M+1 molecular peak is present.Peak is observed due to $C_7H_9N_3O_3$.

166.1 : Peak is observed due to$C_6H_5N_3O_3$.

153.1 :Peak is observed due to $C_6H_5N_3O_2$.

135.1 : Peak is observed due to $C_5H_5N_3O$.112.0 :Base peak is observed due to$C_4H_3N_2O_2$.Thisis Uracil peak.

Table 1: Various Derivatives of Alanine.

Sr . No	Compound Name	M.P	Nitrogen Rule	Rule Of 13		Compound Formula	Base Formula C_nH_{n+r}	Unsaturation Index (U)
				n	r			
1	M_1A	>300°C	Yes	15	11	$C_8H_{10}N_6O_1$	$C_{15}H_{26}$	7
2	M_1C	>300°C	Yes	17	1	$C_8H_{10}N_6O_2$	$C_{17}H_{18}$	14
3	M_1D	>300°C	Yes	15	2	$C_8H_{11}N_3O_3$	$C_{15}H_{17}$	12
4	M_1E	>300°C	Yes	21	10	$C_7H_9N_3O_3$	$C_{21}H_{13}$	11

4. Antimicrobial Activity

In antimicrobial activity, when the compound solution was introduced to sterile petri plate. Then the compound was diffused into the agar media. If the compound was effected against bacteria or fungi had not growth enough to be visible. This is called a zone of inhibition. The size of zone was depended on the concentration of test compound.

The zone of inhibition was along with the rate of compound diffusion. It was used to estimate the bacteria's sensitivity to that suitable antibiotic. Generally, largest zones was correlated with smallest minimum inhibitory concentration (MIC) of antibiotic for that bacteria or fungi.

The production of zone inhibition by the test was compared with the zone produced by known concentration of a standard compound. This all information could be used to chosen appropriate antibiotics or antifungal to combat proper infection.

Bacteria could be divided on the basis of their morphological characteristics like higher and lower bacteria. The higher bacteria were filamentous organisms, few being coated had being certain cells specially for producing diseased in animal or human were known as "Pathogens". The lower bacteria had chiefly unicellular structures, never in the form of coated filaments like Cocci, bacilli.

To test the fungicidal activity of sample, various plant pathogenic organisms were used. This activity was studied at different concentration indifferent pathogenic organism. The agar cup method was used for antifungal activity. This

activity was performed sameas antibacterial activity.

We have used the **Broth Dilution Method** to evaluate the antibacterial activity.

The main advantage of the **'Broth Dilution Method'** for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately sub cultured [before inoculation] by spreading a lapful evenly over a quarter of p[late of medium suitable for the growth of the test organism and put for incubation at 37 ^{0}C OVERNIGHT. The tubes are then incubated overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.
5. The amount of growth from the control tube before incubation [which represents the original inoculum] is compared.

Table 2: Antibacterial Activity of Standard Drugs

Bacteria	**Zone inhibition in mm**				
	Gentamycin	**Ampicillin**	**Chloramphenicol**	**Ciprofloxacin**	**Norfloxacin**
E coli	0.05	100	50	25	10
P.Areuginosa	1	0	50	25	10
S.Aureus	0.25	250	50	50	10
S.Pyogenus	0.5	100	50	50	10

Table 3 : Antibacterial Activityof Compounds

Bacteria	Zone inhibition in mm		
	M_1A	**M_1D**	**M_1E**
E coli	125	100	500
P.Areuginosa	250	250	250
S.Aureus	200	125	125
S.Pyogenus	200	200	125

Table 4: Antifungal Activity of StandardDrugs

Fungi	Zone inhibition in mm	
	Nystatin	Greseofulvin
C.Albicans	100	500
A.Niger	100	100
A.Clavatus	100	100

Table 5: Antifungal Activity of Compounds

Fungi	Zone inhibition in mm		
	M_1A	M_1D	M_1E
C.Albicans	500	500	1000
A.Niger	500	250	250
A.Clavatus	1000	250	250

Result and Discussion

According to observation table.3 Sample contain MIC range 0.001 ml to 0.005 ml constitute 0.01 mg in 10 ml solvent. The activity of M_1A, M_1D, M_1E extract is observed between. 125 mm to 500 mm. against respective strain. At each strain lowest MIC activity observed in standard drugs minimum 0.05 mm and maximum 250 mm. This activity indicate

zone of inhibition against various bacterial strain such as *E.coli, p.areusinasa ,s.aureus and s.pyagenls* of same dilution. The activity of standard drug was given in table 2.

Antibacterial activity of compoundsM_1A, M_1D, M_1E are excellent as compare to the standard drug at same concentration.

According to observation table.5 Sample contain MIC range 0.001 ml to 0.005 ml constitute 0.01 mg in 10 ml solvent. The activity of M_1A, M_1D, M_1E extract is observed between. 250 mm to 1000 mm. against respective strain. At each strain lowest MIC activity observed in standard drugs minimum 100 mm and maximum 500 mm. This activity indicate zone of inhibition against various fungal strain such as *C.Albicans, A.Niger, A.Clavatus* of same dilution. The activity of standard drug was given in table.4.

Antifungal activity of compounds M_1A, M_1D, M_1E are excellent as compare to the standard drug at same concentration.

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